Currently Pending Claims

45. A method for modifying an oligonucleotide, said method comprising:

(a) combining said oligonucleotide with a polynucleotide and a 5'-nuclease, said oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5' portion which does not hybridize to the polynucleotide,

(b) incubating said oligonucleotide, said polynucleotide, and said nuclease under isothermal conditions, whereby a duplex formed by hybridization of the 3' portion of the oligonucleotide to the polynucleotide is in equilibrium with unhybridized oligonucleotide and unhybridized polynucleotide, said isothermal conditions being at or near the melting temperature of said complex, and

(c) while maintaining said isothermal conditions, cleaving said oligonucleotide with said nuclease when said 3'-portion is hybridized to said polynucleotide to provide:

(i) a first fragment including said 5'-portion and no more than one nucleotide from the 5' end of said 3'-portion, and

(ii) a second fragment that is 3' of said first fragment with reference to the intact oligonucleotide, thereby modifying said oligonucleotide, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

46. The method of claim 45, wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

47. The method of claim 45, further comprising incubating a second oligonucleotide under said isothermal conditions with said oligonucleotide, said polynucleotide, and said 5'-nuclease, wherein said second oligonucleotide substantially non-reversibly hybridizes under said isothermal conditions to a site on said polynucleotide that is in the 3' direction from the site at which said oligonucleotide hybridizes.

48. The method of claim 47, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3 °C higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

49. A method for amplifying a signal associated with the presence of a polynucleotide analyte, said method comprising:

(a) providing in combination a polynucleotide analyte, a 5'-nuclease and a molar excess, relative to the concentration of said polynucleotide analyte, of an oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to said polynucleotide,

(b) under isothermal conditions, establishing an equilibrium between said oligonucleotide, said polynucleotide analyte, and a duplex formed by the hybridization of the 3' portion of said oligonucleotide with said polynucleotide analyte, said isothermal conditions being at or near the melting temperature of said duplex,

(c) while maintaining said isothermal conditions, cleaving said oligonucleotide with said 5'-nuclease when said oligonucleotide is hybridized to said polynucleotide to provide,

(i) a first fragment including said 5'-portion and no more than one nucleotide from the 5'-end of said 3'-portion, and

(ii) a second fragment including at least one of said 3' portion and said 3' portion lacking one nucleotide, wherein at least one of said first fragment and said second fragment generates a signal, and

(d) while maintaining said isothermal conditions, maintaining said equilibrium to amplify the amount of at least one of said first fragment and said second fragment and thereby amplifying said signal, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

50. The method of claim 49 further comprising maintaining said equilibrium until at least a 100-fold molar excess of said first fragment and/or said second fragment are obtained relative to the molar amount of said polynucleotide analyte.

51. The method of claim 49 wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaciae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanasomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobaccillus moniliformis, Donvania granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

52. The method of claim 49, further comprising hybridizing a second oligonucleotide to said polynucleotide analyte under said isothermal conditions, wherein said second oligonucleotide hybridizes to a site on said polynucleotide analyte that is in the 3' direction of the site at which said oligonucleotide hybridizes, and wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3°C higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

53. The method of claim 49, wherein said oligonucleotide hybridization sites are contiguous.

54. The method of claim 49, wherein at least one of said first fragment and said second fragment has a label.

55. The method of claim 54, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules,

chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

56. A method for detecting a polynucleotide analyte, said method comprising:

(a) providing in combination a medium suspected of containing said polynucleotide analyte, a molar excess, relative to the suspected concentration of said polynucleotide analyte, of a first oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to said polynucleotide, a 5'-nuclease, and a second oligonucleotide that hybridizes to a site on said polynucleotide analyte in the 3'-direction of the site at which said first oligonucleotide hybridizes,

(b) under isothermal conditions, establishing an equilibrium between a complex formed by the hybridization of the 3' portion of said first oligonucleotide and said polynucleotide analyte, said polynucleotide analyte and said first oligonucleotide, said isothermal conditions being at or near the melting temperature of said complex, and wherein said second oligonucleotide is substantially fully hybridized to said polynucleotide analyte under said isothermal conditions,

(c) while maintaining said isothermal conditions, cleaving said first oligonucleotide when hybridized to said polynucleotide analyte with said 5'-nuclease to provide,

(i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte, and

(ii) a second fragment that is 3' of said first fragment in said first oligonucleotide and which substantially hybridizes to said polynucleotide analyte; and

(d) while maintaining said isothermal conditions, detecting the presence of said first fragment, said second fragment, or said first fragment and said second fragment, the presence thereof indicating the presence of said polynucleotide analyte wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

57. The method according to claim 56, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3°C. higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

58. The method of claim 56, wherein said first fragment and/or said second fragment has a label.

59. The method of claim 58, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

60. The method of claim 56 wherein said polynucleotide analyte is DNA.

61. The method of claim 56, wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said first oligonucleotide that is capable of hybridizing to said polynucleotide analyte.

62. The method of claim 56, wherein said second oligonucleotide hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first oligonucleotide hybridizes.

63. The method of claim 56, wherein said first oligonucleotide has a substituent that facilitates separation of said first fragment or said second fragment from said medium.

64. The method of claim 56 wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaciae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic

Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanasomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobaccillus moniliformis, Donvania granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

65. A method for detecting a polynucleotide analyte, said method comprising:

(a) providing in combination a medium suspected of containing said polynucleotide analyte, a first oligonucleotide at least a portion of which reversibly hybridizes with said polynucleotide analyte under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, a 5'-nuclease, and a second oligonucleotide that hybridizes to a site on said polynucleotide analyte that is in 3' of, and contiguous with, the site at which said first oligonucleotide hybridizes, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3°C higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide,

(b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first oligonucleotide, wherein said first oligonucleotide, when hybridized to said polynucleotide analyte, is cleaved by said 5'-nuclease as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte,

(i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte, and

(ii) a second fragment that is 3' of said first fragment in said first oligonucleotide and which substantially hybridizes to said polynucleotide analyte; and

(c) detecting the presence of said first fragment, said second fragment, or said first fragment and said second fragment, the presence thereof indicating the presence of said polynucleotide analyte, wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaciae, Enteric

bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanasomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobaccillus moniliformis, Donvania granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

66. The method of claim 65, wherein at least one of said first fragment and said second fragment has a label.

67. The method of claim 66, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

68. The method of claim 65, wherein said polynucleotide analyte is DNA.

69. A method for modifying an oligonucleotide, said method comprising:

(a) combining said oligonucleotide with a polynucleotide and a 5'-nuclease, said oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5' portion which does not hybridize to the polynucleotide,

(b) incubating said oligonucleotide, said polynucleotide, and said nuclease under isothermal conditions, and

(c) while maintaining said isothermal conditions, cleaving said oligonucleotide with said nuclease when said 3'-portion is hybridized to said polynucleotide to provide:

(i) a first fragment including said 5'-portion and no more than one nucleotide from the 5' end of said 3'-portion, and

(ii) a second fragment that is 3' of said first fragment with reference to the intact oligonucleotide, thereby modifying said oligonucleotide.

70. The method of claim 69, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

71. The method of claim 69, wherein the amounts of fragments that are formed are larger than the amount of said polynucleotide.

72. The method of claim 69, further comprising incubating a second oligonucleotide, said polynucleotide, and said 5'-nuclease, wherein said second oligonucleotide hybridizes to a site on said polynucleotide that is in the 3' direction from the site at which said oligonucleotide hybridizes.

73. The method of claim 72, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

74. The method of claim 69 wherein said polynucleotide is from a pathogenic organism.

75. The method of claim 69, wherein at least one of said first fragment and said second fragment has a label.

76. A kit for detection of a polynucleotide comprising in packaged combination:

(a) a first oligonucleotide having the characteristic that, when reversibly hybridized under isothermal conditions to at least a portion of said polynucleotide, it is degraded by a 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and (ii) a second fragment that is 3' of said first fragment in said first oligonucleotide and is substantially hybridizable to said polynucleotide,

(b) a second oligonucleotide having the characteristic of hybridizing to a site on said polynucleotide that is 3' from the end of the site at which said first oligonucleotide hybridizes, wherein said polynucleotide is hybridized to said second oligonucleotide under said isothermal conditions, and

(c) a 5'-nuclease.

77. The kit of claim 76, wherein said second oligonucleotide is separated by no more than one nucleotide from the 3'-end of the site at which said first oligonucleotide hybridizes.

78. The kit of claim 76, wherein said first oligonucleotide and said second oligonucleotide are DNA.

79. A method for modifying an oligonucleotide, the method comprising incubating under isothermal conditions the oligonucleotide, a polynucleotide, and a nuclease, wherein the oligonucleotide and the polynucleotide form a complex comprising said oligonucleotide and said polynucleotide in which at least a portion of the oligonucleotide is hybridized to the polynucleotide, wherein the isothermal conditions are at or near the melting temperature of the complex and wherein the oligonucleotide, when the portion is hybridized to the polynucleotide, is cleaved by the nuclease to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than five nucleotides from the 5'-end of the portion and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide, thereby modifying said oligonucleotide, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

80. The method of claim 79 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of the polynucleotide.

81. The method of claim 79 wherein a second oligonucleotide is present during the incubating, wherein the second oligonucleotide hybridizes to a site on the polynucleotide that

is in the 3' direction from the site at which the oligonucleotide is hybridized and wherein the second oligonucleotide is substantially non-reversibly hybridized to the polynucleotide under the isothermal conditions.

82. The method of claim 81 wherein the second oligonucleotide hybridizes to the polynucleotide at a site contiguous with the site on the polynucleotide at which the oligonucleotide hybridizes.

83. The method of claim 82 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of the polynucleotide.

84. A method for detecting a polynucleotide analyte, which comprises:

(a) forming a mixture comprising a sample suspected of containing a polynucleotide analyte, an oligonucleotide and a nuclease,

(b) incubating the mixture at a temperature at which the oligonucleotide reversibly hybridizes to the polynucleotide analyte, wherein the oligonucleotide has a 5' portion which does not substantially hybridizes with the polynucleotide analyte at said temperature and a 3' portion which substantially hybridizes with the polynucleotide analyte at said temperature, thereby forming a polynucleotide complex comprising at least the polynucleotide analyte and the oligonucleotide, wherein the complex serves as a substrate for the nuclease, and wherein during said incubating the nuclease cleaves the oligonucleotide when the oligonucleotide is hybridized to the polynucleotide analyte to continuously produce (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and includes no more than five nucleotides from the 5'-end of the portion which substantially hybridizes to the polynucleotide analyte, and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide analyte, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

85. The method of claim 84 wherein at least one of the first fragment and the second fragment has a label.

86. The method of claim 84 wherein the first fragment includes no more than one nucleotide from the 5'-end of the portion of the oligonucleotide that substantially hybridizes to the polynucleotide analyte.

87. The method of claim 84 wherein the mixture further comprises a second oligonucleotide that substantially fully hybridizes to a site on the polynucleotide analyte that is in the 3' direction from the site at which the oligonucleotide hybridizes and wherein the second oligonucleotide is substantially fully hybridized to the polynucleotide analyte at the temperature.

88. The method of claim 87 wherein the second oligonucleotide hybridizes to the polynucleotide analyte at a site contiguous with the site on the polynucleotide analyte at which the oligonucleotide hybridizes.

89. A method for detecting a polynucleotide analyte, the method comprising:

(a) providing in combination a medium suspected of containing the polynucleotide analyte, a molar excess, relative to the suspected concentration of the polynucleotide analyte, of a first oligonucleotide at least a portion of which is reversibly hybridizes with the polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second oligonucleotide that hybridizes to a site on the polynucleotide analyte that is in the 3' direction of the site at which the first oligonucleotide reversibly hybridizes wherein the polynucleotide analyte is substantially fully hybridized to the second oligonucleotide under the isothermal conditions,

(b) reversibly hybridizing under the isothermal conditions the polynucleotide analyte and the first oligonucleotide, wherein the first oligonucleotide, when hybridized to the polynucleotide analyte, is cleaved by the 5'-nuclease as a result of the presence of the polynucleotide analyte to provide, in at least a 100-fold molar

excess of the polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that is 3' of the first fragment with reference to the intact first oligonucleotide and is substantially hybridizable to the polynucleotide analyte, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

90. The method of claim 89 wherein the first fragment and/or the second fragment has a label.

91. The method of claim 90 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

92. The method of claim 89 wherein the polynucleotide analyte is DNA.

93. The method of claim 89 wherein the first fragment includes no more than 5 nucleotides from the 5'-end of the portion of the first oligonucleotide that is reversibly hybridizes to the polynucleotide analyte.

94. The method of claim 89 wherein the second oligonucleotide hybridizes to the polynucleotide analyte at a site contiguous with the site on the polynucleotide analyte at which the first oligonucleotide reversibly hybridizes.

95. A method for detecting a DNA analyte, the method comprising:

(a) providing in combination a medium suspected of containing the DNA analyte, a first oligonucleotide at least a portion of which reversibly hybridizes with the DNA analyte under isothermal conditions, a 5' nuclease, and a second oligonucleotide that hybridizes to a site on the DNA analyte that is in the 3' direction

from the site at which the first oligonucleotide reversibly hybridizes wherein the DNA analyte is substantially fully hybridized to the second oligonucleotide under the isothermal conditions,

(b) reversibly hybridizing the DNA analyte and the first oligonucleotide under the isothermal conditions, wherein the first oligonucleotide, when hybridized to the DNA analyte, is cleaved by the 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to the DNA analyte and (ii) a second fragment that is 3' of the first fragment with reference to the intact first oligonucleotide and is substantially hybridizable to the DNA analyte, wherein at least a 100-fold molar excess, relative to the DNA analyte, of the first fragment and/or the second fragment is produced, and wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the DNA analyte.

96. The method of claim 95 wherein the first oligonucleotide has a substituent that facilitates separation of the first fragment or the second fragment from the medium.

97. The method of claim 95 wherein first fragment and/or second fragment has a label.

98. The method of claim 97 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

99. The method of claim 95 wherein the second oligonucleotide hybridizes to the DNA analyte at a site contiguous with the site on the DNA analyte at which the first oligonucleotide reversibly hybridizes.

100. The method of claim 95 wherein the first oligonucleotide and/or the second oligonucleotide is DNA.

101. A method for detecting a polynucleotide analyte, the method comprising:

(a) providing in combination a medium suspected of containing the polynucleotide analyte, a first DNA oligonucleotide at least a portion of which reversibly hybridizes with the polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second DNA oligonucleotide that hybridizes to a site on the polynucleotide analyte that is 3' of, and contiguous with, the site at which the first DNA oligonucleotide, reversibly hybridizes, wherein the polynucleotide analyte is substantially fully hybridized to the second DNA oligonucleotide under the isothermal conditions,

(b) reversibly hybridizing under the isothermal conditions the polynucleotide analyte and the first DNA oligonucleotide, wherein the first DNA oligonucleotide, when hybridized to the polynucleotide analyte, is cleaved by the 5'-nuclease as a result of the presence of the polynucleotide analyte to provide, in at least a 100-fold molar excess of the polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and/or (ii) a second fragment that is 3' of the first fragment with reference to the intact first DNA oligonucleotide and is substantially hybridizable to the polynucleotide analyte, wherein said first fragment and/or said second fragment is/are continuously produced under said isothermal conditions, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

102. The method of claim 101 wherein the first fragment and/or the second fragment has a label.

103. The method of claim 102 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules,

chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

104. The method of claim 101 wherein the polynucleotide analyte is DNA.

105. A method for modifying an oligonucleotide, the method comprising incubating the oligonucleotide with a polynucleotide and a 5'-nuclease under isothermal conditions, wherein at least a portion of the oligonucleotide reversibly hybridizes to the polynucleotide under said isothermal conditions and wherein the oligonucleotide, when the portion is hybridized to the polynucleotide, is cleaved by the 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than five nucleotides from the 5'-end of the portion and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide, thereby modifying said oligonucleotide, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

106. A method for producing oligonucleotide cleavage products from an enzyme catalyzed cleavage of the oligonucleotide, the method comprising:

(a) combining, in any order, a polynucleotide, an oligonucleotide having a 3'-portion which substantially hybridizes with the polynucleotide and a 5' portion which does not substantially hybridize with the polynucleotide, and a nuclease, wherein the oligonucleotide, when hybridized to the polynucleotide, forms a polynucleotide complex comprising at least the polynucleotide and the oligonucleotide, the complex serving as a substrate for the nuclease,

(b) incubating the oligonucleotide, the polynucleotide and the nuclease at a temperature at which the oligonucleotide reversibly hybridizes to the polynucleotide, wherein the nuclease cleaves the oligonucleotide when the oligonucleotide is hybridized to the polynucleotide to continuously produce at said temperature (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than 5 nucleotides from the 5'-end of the portion which substantially hybridizes to the polynucleotide, and (ii) a second fragment that is 3' of the first fragment with

reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide, thereby producing oligonucleotide cleavage products.

107. A method for detecting a polynucleotide analyte, which comprises:

(a) reversibly hybridizing an oligonucleotide with a polynucleotide analyte and a 5'-nuclease under isothermal conditions wherein the polynucleotide analyte serves as a recognition element to enable the 5'-nuclease to cleave the oligonucleotide to provide (1) a first fragment that is substantially non-hybridizable to the polynucleotide analyte, and (ii) a second fragment that lies 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide analyte, wherein at least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and

(b) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.